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(54) Title: PHARMACEUTICAL COMPOSITION CONTAINING IL-6, THEIR USES FOR THE TREATMENT OF CONSUMPTIVE THROMBO-HEMORRHAGIC DISORDER

(57) Abstract

The invention provides use of interleukin-6 in the manufacture of a medicament for the treatment or prophylaxis of consumptive thrombohemorrhagic disorder. The invention also provides use of interleukin-6 in the manufacture of a medicament for the treatment or prophylaxis of a dysfunction associated with a reduced level of at least one acute phase protein.

PHARMACEUTICAL COMPOSITION CONTAINING IL-6. THEIR USES FOR THE TREATMENT OF CONSUMPTIVE THROMBO-HEMORRHAGIC DISORDER

This invention relates to the novel use of certain cytokines in the treatment of certain blood disorders, primarily consumptive thrombohemorrhagic disorder.

Consumptive thrombohemorrhagic disorder comprises disseminated intravascular coagulation (DIC), defibrination syndrome and consumptive coagulopathy. A consumptive thrombohemorrhagic disorder is a pathological syndrome, the manifestation of which can in large part be regarded as a consequence of thrombin formation although other features such as blood factor and platelet consumption and fibrinolysis are present. Thrombin catalyses the activation and subsequent consumption of certain coagulant proteins and production of fibrin thrombi or clots. The fibrin thrombus is seen as an indicator of DIC. Microvascular, non adherent thrombi are present in almost all cases of DIC.

The symptoms of consumptive thrombohemorrhagic disorder such as DIC vary with the stage and severity of the consumptive thrombohemorrhagic disorder. Most patients have extensive skin and mucous membrane bleeding and hemorrhage from multiple sites. Occasionally patients have abnormalities in laboratory tests without clinical manifestations. The major manifestations in laboratory tests include thrombocytopenia, prolonged prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) and a reduced fibrinogen plasma level illustrating the consumption of essential coagulation factors. Elevated fibrin degradation products (FDPs or fibrin split products) account for intense secondary fibrinolysis. Other factors such as factors V, VIII and XIII are usually decreased. Such findings can strengthen the diagnosis. In particular lowered factor VIII levels may be a sensitive indicator. However, the major manifestation of DIC, which correlates closely with bleeding, is the

faced with the task of balancing the patient's requirements for several substances in order to restore normal hemostasis.

Surprisingly, we have discovered that the administration of interleukin-6 (IL-6) can be used beneficially in combatting consumptive thrombohemorrhagic disorder such as DIC in that it not only increases blood platelet count but simultaneously elevates the level of plasma fibrinogen and inhibits fibrinolysis and thrombin formation. It also induces an elevation of plasma acute phase proteins, such as alpha-1 antitrypsin and alpha-2 macroglobulin which are known natural inactivators of plasmin and thus inhibitors or fibrinolysis.

One aspect of the present invention provides use of interleukin-6 in the manufacture of a medicament for the treatment or prophylaxis of consumptive thrombohemorrhagic disorder, preferably disseminated intravascular coagulation.

The invention further includes a method of treatment or prophylaxis of a human or animal subject suffering from or at risk to consumptive thrombohemorrhagic disorder, preferably disseminated intravascular coagulation wherein an effective dose of interleukin-6 is administered to said subject.

Also, the invention additionally provides use of IL-6 in the manufacture of a medicament for the treatment or prophylaxis of a dysfunction associated with a reduced level of at least one plasma acute phase protein.

This use of IL-6 is ideally suited to the management of DIC since it not only meets the requirements stated above but also increases the blood platelet count.

The expression "interleukin-6" and the term "IL-6" are both intended to encompass natural, synthetic and recombinant forms of the polypeptide as well as derivatives thereof. IL-6 has been characterised and discussed for example in M. Revel, Experientia 54: 549-557 (1989). Preferably, recombinant human IL-6 (hrIL-6) is used.

c) Primary fibrinolysis, in which the mechanisms that localise fibrinolysis are overwhelmed by release of plasminogen activators, leading to bleeding; and

d) Microangiopathic thrombocytopenia, in which platelet microthrombi are widespread, leading to depletion of platelets, ischemic necrosis of tissues, and microangiopathy changes in red cells.

The initial stages of DIC may not be readily apparent to the clinician because, for example, the cause of DIC, e.g. infection, may mask the early stages in the course of the disorder. However, the disorder may gain momentum rapidly and assume importance beyond that of the initiating stimulus.

Intrinsic and extrinsic coagulation systems are activated in DIC with resulting local and general escape of thrombin into the circulatory system. Alterations of any of the components of the vascular system, namely vessel wall, plasma proteins, and platelets, can result in a consumptive disorder. Endothelial damage, as mentioned above relates to those disease states which specifically injure the endothelium, with resultant kallikrein-kinin activation i.e. intrinsic coagulation. Tissue injury on the other hand liberates tissue factors and refers to those disease states in which procoagulant material, e.g. tissue thromboplastin, acts locally or is released into the circulation i.e. extrinsic coagulation. The intrinsic and extrinsic coagulation systems lead to the formation of an enzymic complex (factor Xa, factor V, calcium and phospholipids), which transforms prothrombin (factor II) into thrombin.

The consumptive processes of DIC reflect the multiple actions of thrombin. Thrombin proteolytically cleaves fibrinopeptides from fibrinogen to produce fibrin monomers which either combine with fibrinogen to form soluble complexes or polymerize to form fibrin thrombi. The fibrin thrombi often cause microvascular occlusions which lead to local hypoperfusion, and even ischemia, infarction and necrosis. The fibrin formation initiated by thrombin decreases plasma fibrinogen concentration.

induced, whether it is triggered by contact i.e. intrinsic coagulation system or by tissue injury i.e. extrinsic coagulation system. The balance between these two proteases, i.e. thrombin and plasmin, determines whether the clinical picture is characterized by thrombosis, organ ischemia and bleeding (thrombin predominance) or predominantly by bleeding (thrombin and plasmin action). Alpha-1 antitrypsin as well as alpha-2 macroglobulin naturally inactivate plasmin. Both alpha-1 antitrypsin and alpha-2 macroglobulin are acute phase proteins and others of the group include C3c complement, transferrin, haptoglobulin acid α-1 glycoprotein, ceruloplasmin and C-reactive protein.

The involvement of IL-6 in fibrinolysis is further demonstrated by our observation that on injection of IL-6, both tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) are released. This suggests stimulation by IL-6 of the endothelial cells. t-PA levels rise about four fold, which is within the range observed in healthy subjects, for example after exercise, but the rise in PAI-1 levels is about 30-fold.

It will be clear from the foregoing that it is desirable in the management of DIC to increase the blood platelet count, elevate the plasma fibrinogen level, induce a prolonged thrombin time and increase inhibitors of fibrinolysis and fibrinogenlysis (alpha-2 macroglobulin and alpha-1 antitrypsin which are plasma acute phase proteins); all of which are produced by administration of IL-6.

Medicaments comprising IL-6 may be administered by the oral, rectal, intranasal, transdermal and parenteral routes, the latter being preferred. The proposed dosage is preferably 35 to 350 μg of active substance per dose, low doses being appropriate for infusion or injection and higher doses being appropriate for other forms of administration. The recommended dose for intravenous application is from 0.5 to 30 μg/Kg/day, preferably from 1 to 10 μg/Kg/day. Where the active substance is

cyclamate, glycerine or sugar and a flavour-enhancing agent, e.g. a flavouring such as vanillin or orange extract. They may also contain suspension adjuvants or thickeners such as sodium carboxymethylcellulose, wetting agents, e.g. condensation products of fatty alcohols with ethylene oxide, or preservatives such as p-hydroxy-benzoates.

Solutions for infusion or injection may be prepared in conventional manner, .e.g. with the addition of preservatives such as p-hydroxybenzoates or stabilisers such as alkali metal salts of ethylene-diamine tetraacetic acid and may then be transferred into fusion vessels, injection vials or ampoules. Alternatively, the compound for injection may be lyophilised either with or without the other ingredients and be solubilised in a buffered solution or distilled water, as appropriate, at the time of use. Bolus intravenous injections may be given.

Capsules containing the active substances or combinations of active substances may be prepared, for example, by mixing the active substances with inert vehicles such as lactose or sorbitol and encapsulating them in gelatine capsules.

Suitable suppositories may be prepared, for example, by mixing with carrier substances provided for this purpose, such as neutral fats or polyethylene glycol or derivatives thereof.

The compound may be mixed with a polylactide or a glutamic acid based copolymer to provide an implantable sustained release delivery system, as described respectively in US 377919 and by K. R. Sidman et al (J. Membrance Sci. 1980 7 277-291).

The invention will now be described by way of illustration only with reference to the following Examples and Figures.

Hermatological examinations

Blood samples were drawn under general anaesthesia (ketamine 5 mg/kg) from the posterior saphena vein. Complete blood cell counts (WBC, RBC, hematocrit, hemoglobin) were performed using a Coulter counter equipped with a veterinary kit and differential white blood cell counts were performed on smear preparations stained with May-Grünwald-Giemsa.

Blood chemistry

A variety of blood chemistry tests were performed, which monitored, for example acute phase proteins such as α1-antitrypsin, and α2-macroglublin.

Haemostasis was monitored inter alia by activated partial thromboplastin time (APTT or kaolin-cephalin time), thrombin time and fibrinogen quantative tests.

The kaolin-cephalin clotting time (or partial thromboplastin time (PTT) and APTT) was used to evaluate the intrinsic system. This is a clotting time of plasma, free of Ca^{++} and poor in platelets, in the presence of cephalin (a substitute for platelet factor III extracted from tissue) and of kaolin (a clay-like substance) that activates under standardized conditions factor XIII. The test is then a measure of factors XII, XI, X, IX, VIII, V, II and I. The normal activated partial thromboplastin time in baboons is of the order of 35 seconds.

Thrombin time was determined on the basis that normal plasma clots in a definite and constant time in the presence of a known quantity of thrombin. The thrombin time is longer in case of hypofibrinogenemia and if there is antithrombin in plasma.

The quantitative assay for fibrinogen uses a clotting time of a diluted plasma in the presence of an excess of thrombin. The time required to clot is directly related to the amount of plasmatic fibrinogen.

Fourteen new male baboons weighing between 20 and 25 kg were randomly allocated to three groups. One group of 6 irradiated subjects ("irradiated treated" group or IT) received 10 µg/kg/day of rhIL-6 into two daily subcutaneous injections for 13 consecutive days. This dosage was deemed optimal based on efficacy and tolerance features generated during the preliminary dose finding study in normal monkeys. One group of 6 irradiated subjects ("irradiated non treated" group or INT) received the vehicle only in an identical schedule. One group of 2 subjects ("sham irradiated" group of NIT) received 10 µg/kg/day in an identical fashion and served as the rhIL-6 bioactivity control. All treatments (rhIL-6 or vehicle) always started one day (day 1) after the irradiation date (day 0).

RESULTS IN NON-IRRADIATED BABOONS INJECTED WITH IL-6

Clinical tolerance

The follow up of clinical symptomatology was done every day for at least forty days after the onset of treatment. No significant modifications of body weight, food consumption, body temperature and behaviour were observed during the study duration. No signs of general or local (at the injection sites) intolerance were noted. No deaths in either group were recorded.

Hematological examinations

Human recombinant IL-6 induced a significant increase in blood platelet count generally starting after 4 to 5 days of treatment for all doses tested. Time to the thrombocytosis varied from day 8 up to 13 after the treatment onset and was not related to dose. Although the number of individuals in each dose group was too small to perform a statistical analysis, a dose dependent response trend was observed up to 10 µg/kg/day for the

Hematological examinations

Recombinant hIL-6 significantly attenuated radiation induced thrombocytopenia and accelerated platelets recovery.

The neutronic irradiation induced a deep thrombocytopenia in both groups. The time to nadir defined as the number of days from day 0 to the nadir was significantly different with a mean time of 7.7 days ± 0.8 corresponding to 7 full day of rhIL-6 therapy in the treated group versus a mean time of 12.8 days ± 1.9 in the control group (p=0.003). Moreover the mean time to return at least to the baseline value (calculated as the mean platelet count of the twelve baboons, before irradiation) was significantly shorter in the treated group, 17.3 days ± 5.2 verses 25.0 ± 2.2 in the untreated group (p=0.003). There was a significant increase in platelet count above normal values (peak 559,000/mm^3, p=0.03) for a few days during the recovery phase of the irradiated treated animals compared to the spontaneous recovery slope of the non treated group. As expected, the "sham irradiated" treated controls displayed a considerable thrombocytosis, confirming the data observed during the dose finding study.

In contrast, rhIL-6 therapy neither attenuated the intensity of the radiation induced leukopenia nor accelerated its recovery.

Hemostasis

The thrombin time duration increased beyond normal values in groups receiving rhIL-6 (either IT or NIT). There was a statistically significant difference (in average from day 2 to day 14) between the IT and INT groups (see below):

mean Thrombin Time (sec)	IT	INT
	28.9	18.7

(NIT), with a very progressive return to baseline values after treatment discontinuation.

C reactive protein: all groups of animals displayed a significant increase. However, this elevation was transient in the irradiated non-treated animals (INT), although it lasted during the whole rhIL-6 treatment period for the two other groups. Normalisation was rapidly occurring after treatment discontinuation.

Acid α-1-glycoprotein and Haptoglobulin: a clear increase was observed in animals receiving rhIL-6 (IT and NIT), followed by a progressive normalisation.

α-1-Antitrypsin: a clear increase was observed in animals receiving rhIL-6 (IT and NIT), followed by progressive normalisation.

α-2-Macroglobulin: an increase was observed in all groups of animals. The elevation was transient in the INT group, although it lasted during the whole rhIL-6 treatment period for the two other groups. Normalisation occurred rapidly after treatment discontinuation.

Haemostasis

The most striking feature is the prolongation of the thrombin time and to a lesser extent the duration increase of the APTT. The results suggest a disturbance of fibrin formation which may be linked to the considerable elevation of fibrinogen induced by rhIL-6 in these same animals. However, irradiated non rhIL-6 treated animals (INT) which also displayed a fibrinogen increase although less pronounced, did not show thrombin time perturbations.

It must be stressed that full normalisation of coagulation parameters as well as fibrinogen occurred after treatment discontinuation.

conditioned to 23°C with a relative humidity of 60%. They were fed with commercial primate chow and fresh fruits and tap water ad libitum. Animals were kept under anesthesia (Ketamine 7 mg/kg) during injection and at each blood collection.

Blood collection

Blood was collected from the posterior saphenous vein by clean venepuncture and mixed in precooled tubes without anticoagulant for serum (acute phase proteins) or with anticoagulant (citrate (most assays) or citrate containing a protease inhibitor mixture for fibrinopeptide A or plasmin-antiplasmin complexes Stago).

Assays

All reagents and assay kits have been tested in preliminary experiments for their suitability to assay the respective parameters in baboons.

Coagulation assays

The routine overall coagulation tests: the prothrombin time, the activated partial thromboplastin time and the thrombin time tests were performed using reagents obtained from Diagnostica Stago (Asnières, France). Functional antithrombin III concentrations were measured by a chromogenic substrate technique (Stachrom ATIII, Diagnostica, Stago) and antigen concentrations by nephelometry (Behring, Frankfurt, Germany). Prothrombin fragment 1+2 concentrations were quantified by ELISA (enzygnost 1+2, Behring). Fibrinopeptide A concentrations were measured in plasma after removal of fibrinogen by bentonite adsorption using a competitive enzyme-linked immunoassay (Asserachrom FPA, Diagnostica, Stago). Thrombin-antighrombin III complex concentrations were determined by ELISA (enzygnost TAT micro, Behring). D-dimer concentrations were determined by ELISA (Asserachrom D-Di, Stago)

RESULTS

Effect of a single subcutaneous injection of rh-IL-6 on clinical and hematological parameters

No significant modifications of body weight, food consumption, body temperature or behaviour, signs of general or local (at the injection site) intolerance nor clinical signs of coagulation disorders were observed. No animal loss was recorded in either group. In IL-6 treated baboons platelet counts started to increase after 2-3 days and a maximal increase of 65% was observed around day 7. Variations of platelet counts in the control group were minimal. Hematocrit and hemoglobin values decreased in both the vehicle and IL-6 treated baboons to a minimum of 85% of pre-injection values, which was attained at day 4 to 7 and normalized thereafter.

Interleukin 6 concentrations

Injection of a single subcutaneous dose of 100 µg/kg of rh-IL-6 led to a rapid increase of plasma concentrations of IL-6 that persisted for 24 h (Fig. 1). Peak concentrations of up to 50 ng/ml were obtained at 3h, whereafter IL-6 concentrations gradually declined to 1.3 ng/ml at 24h corresponding to a terminal half life of 3-4 h. In the vehicle injected baboons IL-6 concentrations remained below detection limit (<0.1 ng/ml) throughout the study.

Acute phase response

To confirm that recombinant glycosylated human IL-6 is functional in baboons we measured serum concentrations of several acute phase proteins. C-reactive protein concentrations increased up to eightfold with a maximum at day two after r-hIL-6 injection (Fig. 2A). Alpha-1-antitrypsin concentrations started to increase after a delay of 6h and attained at day 2 maximal values that

concentrations increased after a delay of 3h and attained after 6 to 8 h maximal values that were up to fourfold higher than in the controls, whereafter t-PA concentrations gradually returned to normal (Fig. 10). PAI-1 concentrations increased with a pattern similar to that of t-PA, reached a maximum after 6-8 h that was thirtyfold higher than in the controls and returned to normal within 24 h (Fig. 11).

Plasma concentrations of plasmin-antiplasmin complexes did not change after injection of IL-6 (not shown).

Legends to Figures

Figure 1

Sequential changes (mean ±SEM) of t-PA concentrations in IL-6 injected (closed circles) and control (open circles) baboons.

Figure 2

Sequential changes (mean ±SEM) of PAI-1 concentrations in IL-6 injected (closed circles) and control (open circles) baboons.

9. A method as claimed in claim 8 in which the interleukin-6 is administered intravenously at a dose level of 0.5 to 30 μg/kg body weight/day.

10. A method as claimed in claim 8 in which the interleukin-6 is administered parenterally or by a delayed release formulation at 0.02 to 1.25 μg/kg body weight/ hour.

FIG. 2C

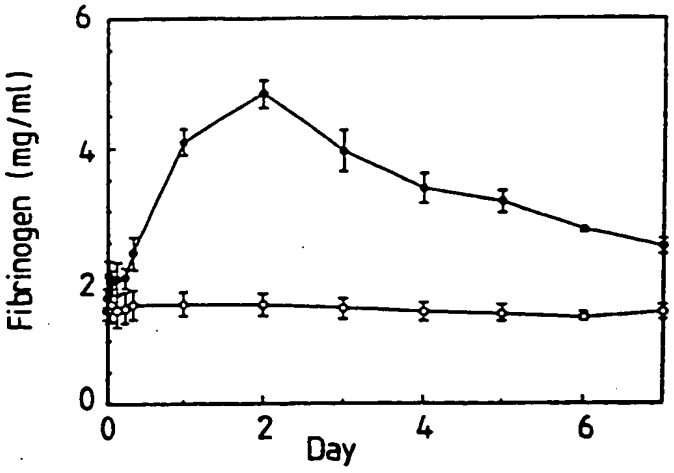


FIG. 2D

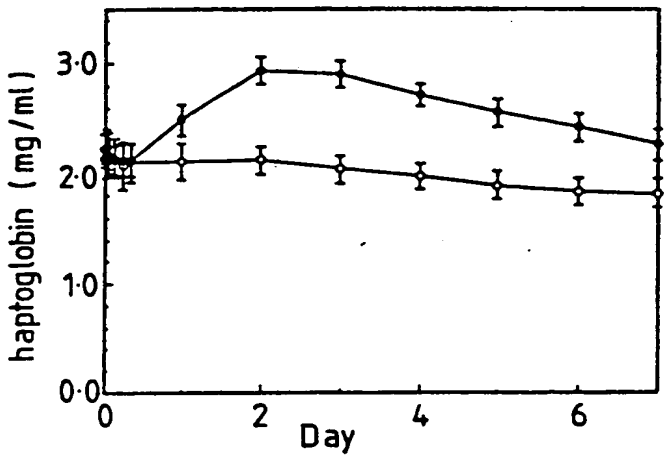


FIG. 2E

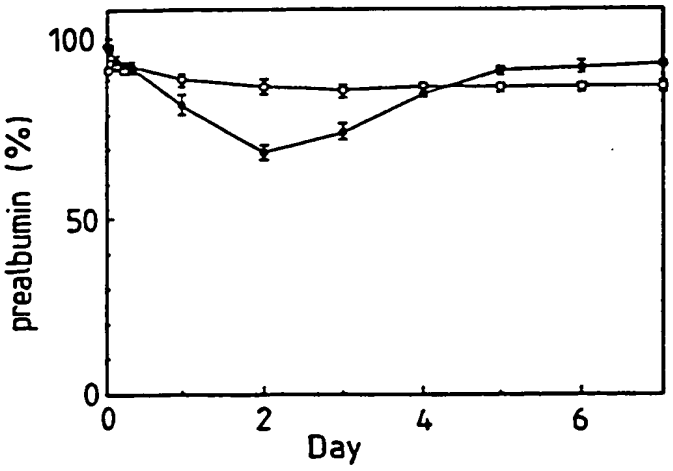


FIG. 3C

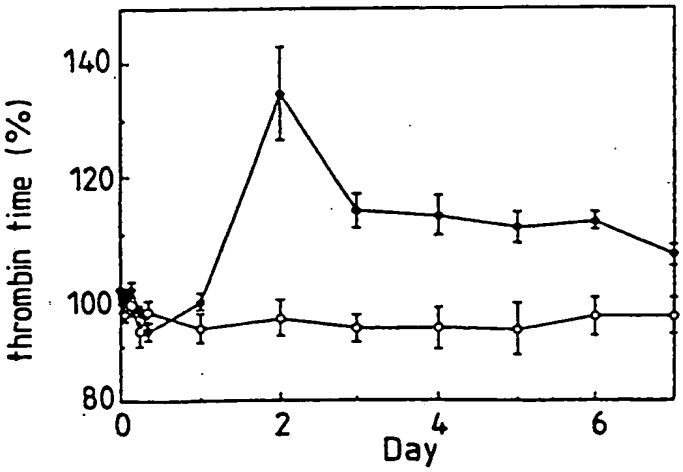


FIG. 4

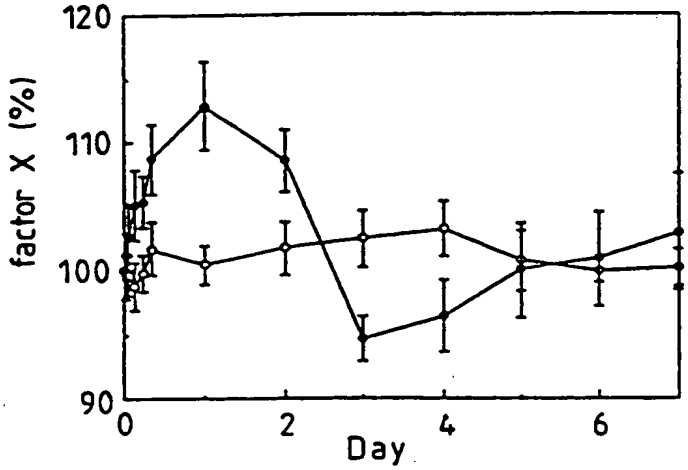


FIG. 5

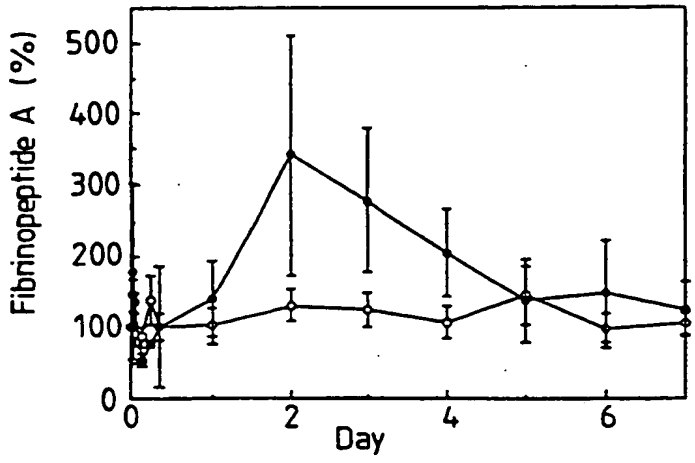


FIG. 7

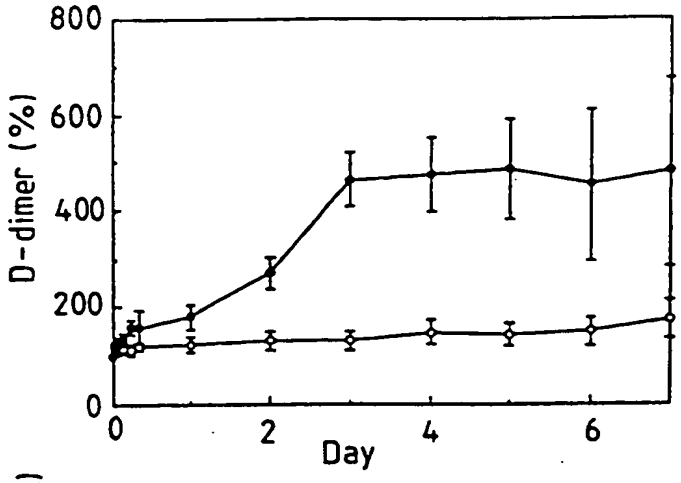


FIG. 8

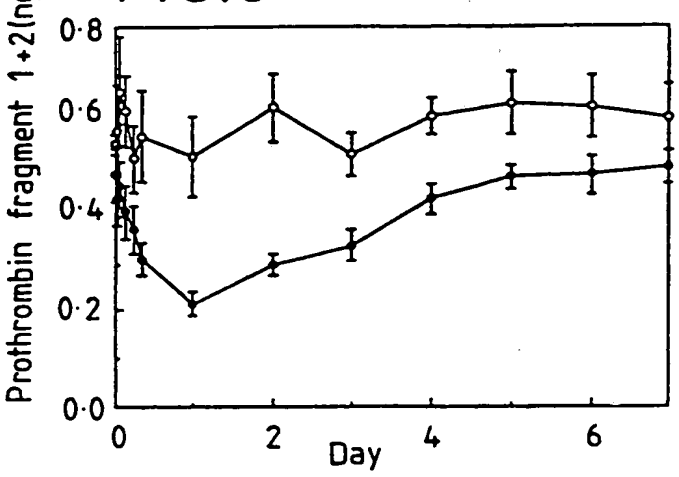
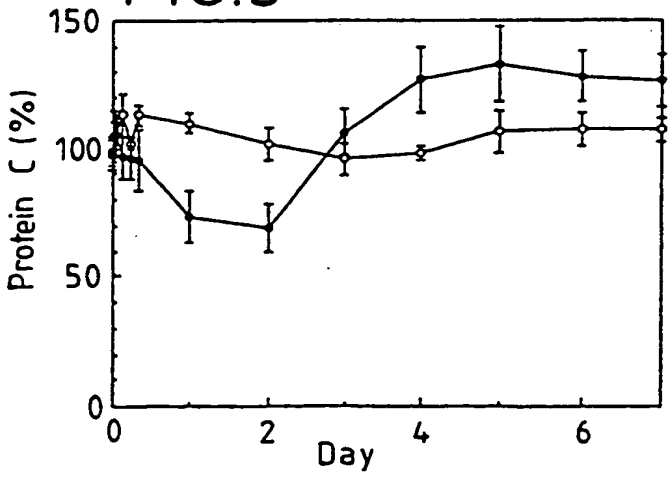


FIG. 9



INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 93/01790

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	FEBS LETTERS. vol. 232, no. 2, May 1988, AMSTERDAM NL pages 347 - 350 J. V. CASTELL ET AL 'Recombinant human interleukin-6 (IL-6/BSF-2/hsf) regulates the synthesis of acute phase proteins in human hepatocytes' * see the whole document especially table 1 * ---	3
P,X	WO,A,9 306 840 (TORAY INDUSTRIES, INC.) 15 April 1993 & EP,A,0 560 998 (TORAY INDUSTRIES, INC.) 22 September 1993 see page 1, line 9 - line 16 see page 3, line 6 - line 11 see page 4, line 7 - line 13 see examples 11-13 --- -/--	1-4,8

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 SEPTEMBER 1993	11 -10- 1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT FFICE	LE CORNEC N.D.R.

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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

EP 9301790
SA 76503

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9306840	15-04-93	EP-A-	0560998	22-09-93
WO-A-9002570	22-03-90	AU-A-	4310889	02-04-90
		EP-A-	0408677	23-01-91
		JP-T-	3501262	22-03-91
EP-A-0378171	18-07-90	JP-A-	3101624	26-04-91
		US-A-	5126325	30-06-92
WO-A-9208493	29-05-92	US-A-	5188828	23-02-93
EP-A-0363083	11-04-90	AU-B-	620451	20-02-92
		AU-A-	4248489	12-04-90
		JP-A-	3072429	27-03-91